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Early Postnatal Benzo(a)pyrene Exposure in Sprague-Dawley Rats Causes Persistent Neurobehavioral Impairments that Emerge Postnatally and Continue into Adolescence and Adulthood

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Previous studies have demonstrated that benzo(a)pyrene (BaP) may disrupt the development of key biological systems, thus leaving children more vulnerable to functional impairments in adulthood. The current study was conducted to determine whether neurotoxic effects of postnatal BaP exposure on behavioral performance persist in juvenile and young adult stages. Therefore, neonate Sprague- Dawleypups were given oral doses of BaP (0.02, 0.2, and 2 mg/kg/day) continuing through a period of rapid brain development (on postnatal days [PNDs] 5–11). Further, developmental milestones and behavioral endpoints assessing sensory and motor maturation were examined. Also, in this study, Morris water maze and elevated plus maze were used for evaluating the cognitive function and anxiety- likebehavior. Our results showed that there was altered ontogeny in a few measures of neuromotor development; however, other developmental milestones and sensory responses were not altered significantly. Moreover, the locomotor activity deficit in BaP-treated pups was evident at PND 36 and was most pronounced in the PND 69. Also, exposure to BaP during early postnatal development had an adverse effect on adult rats (PND 70) in the elevated plus maze, and the swim maze suggests that low doses of BaP impair spatial learning functions at adult test period. In contrast, BaP exposure had no evident effect on behaviors in these two mazes for adolescent animals. These data clearly indicate that behavioral impairments resulting from postnatal BaP exposure are potentially long- lastingand may not be apparent in juveniles, but are present in young adulthood.

Key Words: **benzo(a)pyrene; neurodevelopment; neurotoxicity; postnatal exposure; behavioral alterations; aging; rats.**

Benzo(a)pyrene (BaP) is a member of the prototypical polycyclic aromatic hydrocarbon (PAH) family and has been studied most extensively for its toxic effects. BaP exposure is associated with a wide array of adverse effects in experimental animals, including carcinogenicity, teratogenicity, developmental toxicity, etc. (ATSDR, 1995). Although BaP is ubiquitous in the environment, there is a poor understanding regarding its neurotoxic effects. Of great concern is the knowledge that the central nervous system may be highly susceptible to damage by BaP, and BaP is able to cross the placenta during pregnancy (Das *et al.*, 1985; McCabe and Flynn, 1990).

In recent years, there has been an increasing awareness that fetuses and young children may be more susceptible than adults to the adverse effects of environmental pollutants. However, few specific investigations have been conducted to evaluate the developmental effects of exposure to PAHs in children. Children may have the greatest exposure to BaP from multiple sources such as breast milk, food sources, and ambient air. Moreover, children tend to have greater PAH exposure than adults to contaminated soil in areas where PAHcontaminated soil from industrial contamination may be present because of childspecific behavior patterns, particularly hand-to-mouth activity and more time spent close to and on the ground. Indeed, recent studies have reported that exposure to environmental PAHs (with a range from 1.8 to 272.2 ng/m^3) may adversely affect children's IQ and cognitive development, with potential implications for school performance (Edwards *et al.*, 2010; Perera *et al.*, 2006, 2009). In addition, another study carried out in the Czech Republic has demonstrated that airborne exposure to high levels of PAHs (>15 ng/m^3) increased the incidence of intrauterine growth retardation (Dejmek *et al.*, 2001).

PAHs are ubiquitous and consistently present in our environment and are routinely found in ambient air, food, water, and sediment. The average total daily intake of PAHs by a member of the general U.S. population has been estimated to be 0.207 μg from air, 0.027 μg from water, and 0.16–1.6 μg from food (ATSDR, 1995). In the soil, the levels of PAHs are

in the range of 5–100 μg/kg in unpolluted areas; however, close to highways, the concentrations of PAHs are found in the range of 2–5 mg/kg. Also, the concentrations of PAHs in food products are usually in the range between 0.002 and 0.9 mg/kg (ATSDR, 1995). Taking these data together, the doses 0.02, 0.2 and 2 mg/kg of BaP were used in the present study. Also, these doses have been studied in previous studies (Bouayed *et al.*, 2009; Grova *et al.*, 2007).

The mammalian brain undergoes a period of rapid brain growth, which is called the brain growth spurt (BGS). In the human, the BGS occurs perinatally, spanning from the last 3 months of pregnancy through the first 2 years of life (Dobbing and Sands, 1973, 1979). During this period, the neonate and/or toddler may be directly exposed to xenobiotics (Thomas *et al.*, 2008; Viberg, 2009). However, in rats and mice, the BGS occurs in the neonate, spanning the first 1–2 weeks of life. This period is characterized by the maturation of axonal and dendritic outgrowth and the establishment of neuronal connections. Also during this critical period, animals acquire many new motor and sensory abilities (Kolb and Whishaw, 1989). Children may not only have the low levels of BaP but may also incur the greatest risk, as BaP may interfere with the development of key biological systems, leading to long-term functional impairments. There is a growing literature of animal studies that shows subtle changes in motor and cognitive function following acute or repeated perinatal or lactation exposure to BaP (Bouayed *et al.*, 2009; Edwards *et al.*, 2010; McCallister *et al.*, 2008; Wormley *et al.*, 2004). Therefore, the observation that exposure to BaP during development alters adult behavior has caused concern.

Several studies examining acute subchronic exposure of mice or rats to BaP have shown alterations in motor activity and learning and memory in adult offspring (Bouayed *et al.*, 2009; Grova *et al.*, 2007; Xia *et al.*, 2011). Recently, in a report from McCallister *et al.* (2008), evidence was presented that repeated treatment of pregnant Long-Evans rats with 300 μg/kg BaP by oral gavage resulted in a strong negative effect on brain function in offspring progeny. In another study, 25 and 150 μg/kg doses of BaP were given to adult rats on gestation days 14–17; these treatments caused the alterations at the glutamate receptors in the offspring (Brown *et al.*, 2007). Also, inhalational exposure to BaP at 25, 75, or 100 μg/m^3 resulted in the dose-related reductions in embryonic survival, associated with significant reductions in plasma prolactin concentration at all stages of lactation (Archibong *et al.*, 2001). Moreover, in humans, direct effects of BaP on various cognitive abilities such as long-term memory have been demonstrated (Perera *et al.*, 2005; Rauh *et al.*, 2004). These results showed that perinatal exposure to BaP could induce adverse biological effects for the development of fetus, infant, and newborns. However, unfortunately, these investigators have not examined the effects of BaP on other endpoints during a critical phase of neonatal brain development. In addition, to our knowledge, there are no published studies from other laboratories confirming or refuting behavioral effects following the postnatal day (PND) dosing regimen. Also, to date, there is no applicable report on the behavioral impact of repeated oral postnatal exposures of neonates to BaP. Thus, this study was designed to determine whether developmental exposure of Sprague-Dawley (SD) rats to BaP during BGS would produce any changes in behavioral tests at both the pre- and postweanling ages.

The current study examined developmental milestones in SD rats exposed to a repeated oral dose of BaP on PNDs 5–11. BaP was administered in doses (0.02, 0.2, and 2 mg/kg) that correspond to levels of environmental contamination (ATSDR, 1995). Also, the oral route was selected for this study, which would be more relevant to environmental-exposure situations. Furthermore, behavioral endpoints assessing sensory, motor maturation, anxiety-like behavior, and spatial learning and memory were investigated during pre- and postweanling ages.

MATERIALS AND METHODS

Animals

Sexually mature female (weighing 200–220 g) and male (weighing 310–340 g) SD rats aged 8 weeks obtained from Chongqing Medical University Lab Animal Center were used in this study. The animals were maintained on a standard 12-h light/dark cycle, at a constant temperature (22 C ± 2 C) and a relative humidity of 55 ± 10% with free access to tap water and standard laboratory rodent food. After a 7-day period of acclimatization, virgin female rats were mated with virgin males (2:1) overnight and were examined the following morning for copulatory plugs. The day on which a vaginal plug was present, the pregnant female rats were singly housed in their own polycarbonate cages (wire tops) with shaved wood bedding. The pregnant rats were checked for birth twice daily (0800 h and 1800 h). Day of delivery was considered PND 0, and the pups born during the night were assigned PND 0 when checked at 0800 h.

After parturition, the litters were culled and maintained at eight (four males and four females when possible) during behavioral testing in the preweanling ages to ensure similar nutritional availability in the litters. Previous study has demonstrated that this culling procedure has no measurable effect on subsequent brain or behavior development (Agnish and Keller, 1997). Each rat received an individual number identified with a nontoxic ink marking on their feet. On the day after birth, all pups were randomized (randomizer.org) within treatment groups and redistributed to the nursing dams. For all tests in this study, there were a total of 10 males and 10 females used for behavioral testing for each treatment group, with no more than 1 male and 1 female from each litter. Thus, pups from 40 litters were used for behavioral experiments.

Cageside observations of maternal behavior (retrieval, nursing, and nesting) were made on all test days before weaning. In addition, dams were rotated among litters every 2–3 days to distribute any maternal caretaking differences randomly across litters and treatment groups. Pups were weaned on PND 21, and these weaned pups were originally housed in same-sex and same-exposure groups of five per cage (cage size: 46 × 34 × 20 cm). All animal care and experimental procedures were conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals of Chongqing Medical University.

Chemical and Treatments

Because of its poor water solubility, BaP (purchased from Sigma Chemical Co., St Louis, MO; Lot No.: 090M1400V) stock solution was prepared by mixing the compound with peanut oil to provide consistent absorption and

sonicating for 30 min at 40 C. The daily BaP dosage was determined individually for each rat based on body weight. BaP was administered to rat pups every day by oral gavage at a volume of 3 ml/kg of body weight beginning on PND 5 and continuing through PND 11. Pups were treated between 1300 h and 1600 h. The treatment groups were as follows: (A) control group, which was administered the peanut oil only; (B) 0.02 mg/kg BaP-exposed group; (C) 0.2 mg/kg BaPexposed group; and (D) 2 mg/kg BaP-exposed group. These doses of BaP were selected to be comparable with the daily doses that each human received (ATSDR, 1995).

Behavioral Tests Battery

A battery of developmental/observational tests, which included sensorimotor reflexes, neuromotor behaviors, and physical developmental landmarks, were used during the preweaning period (PNDs 12, 14, 16, 18) and also as adolescents or adults (PNDs 35, 36 and PNDs 70, 71, respectively). The behavioral tests were conducted as described in Table 1. All behavioral testing was carried out during the dark phase of the light/dark cycle (1900 h–2300 h), the most active period, but in lighted environments so that the rats could access the visual cues necessary to perform the tasks. The behavior tests were performed in a quiet room at 40 ± 5 dB. Multiple distant cues around the experiment room were kept in the same location during the behavior tests. Each pup was observed individually, then immediately returned to its home cage. Care was taken to minimize the duration of separation from the dam. The order of testing litters was randomized on each day. The animals were tested under the same temperature conditions as their housing conditions. Tests were performed on 10 rats per sex per treatment group (80 animals in total), with the tests conducted during adolescence and adulthood. In the present study, one male and one female pup from each litter were randomly selected for behavioral testing. Each rat was behaviorally tested only once as either an adolescent or adult. All 80 rats were examined on the mentioned above battery tests and weighed once per day to check for their growth. All tests were conducted by investigators who were unaware of the treatment of each rat, and the order of testing was counterbalanced across treatment groups.

Developmental Landmarks

The emergence of physical maturation landmarks was noted each day, including incisor eruption (the first appearance of upper incisors), eye opening (when both eyelids were completely separated), development of fur (the entire body appeared to be covered in white fur), testes descent (testis descent into the scrotum), and vaginal opening. All pups in each litter were assessed every day scheduled even after attaining each milestone.

Neonatal Sensory and Motor Development

Surface righting reflex test. Pups were gently removed from the litter and placed on a warming pad (33 C). Pups were timed from the moment of being placed in supine position until it had righted itself and all four feet were in contact with the surface. The rats were tested one trial per day. All measures were performed between 1900 h and 2300 h on PNDs 12, 14, 16, and 18.

Negative geotaxis test. Pups were timed for completing a 180 turn when placed in a head down position on a 25 inclined surface. Latency to rotate 180 on the inclined surface was measured during a 120s test. If the pup fell, crawled off the plane, or made no movement, the rat was considered to have failed the task, and a maximum time of 120 s was assigned. Pups were given one trial per day. Pups were tested for negative geotaxis between 1900 h and 2300 h on PNDs 12, 14, 16, and 18.

Cliff aversion test. Pups were placed with their heads and forepaws over the edge of a table. The latency to retract their body (pup turned or crawled away) from the edge was recorded. Pups were given one trial per day. Each rat was tested between 1900 h and 2300 h on PND 12 before eye opening.

Forelimb grip strength test. The grip test was used to measure the maximal muscle strength of forelimbs as a primary phenotype screen. The animal was then placed on a 7.5 × 7.5 cm wire mesh screen that was quickly inverted. The torso of pups was kept horizontal and was pulled back steadily until the grip was released down the complete length of the grid. The type of gripping (front paws only vs. front and back paws) was recorded as well as the length of time the rat was able to hold on the bar unaided before dropping. The ability of the pup to hang onto or climb over the edge of the inverted screen was recorded during three 60s trials each day. The grid was cleaned with 50% ethanol before testing each cage of rats. Each rat was tested between 1900 h and 2300 h on PND 12.

TABLE 1
Behavioral Test Schedule (Age of Testing)

Behavioral tests	PND
Growth and reflex	
Eye opening	Assessed every day after attaining each milestone
Incisor eruption	
Development of fur	
Testes descent	
Vaginal opening	
Surface righting reflex test	PNDs 12, 14, 16, 18 (track 1)
Negative geotaxis test	PNDs 12, 14, 16, 18 (track 2)
Cliff aversion test	PND 12 (track 1)
Forelimb grip strength test	PND 12 (track 2)
Openfield test	PNDs 18 (track 1), 20 (track 2), 34 (track 3), 69 (track 4)
Wean with litter	PND 21
Elevated plus maze	PNDs 35 (track 3), 70 (track 4)
Morris water maze	PNDs 36 (track 3), 71 (track 4)

Note. Track 1: 1♀, 1♂ from each litter were tested for surface righting reflex test, cliff aversion test, and openfield test (PND 18). Track 2: 1♀, 1♂ from each litter were tested for negative geotaxis test, forelimb grip strength test, and openfield test (PND 20). Track 3: 1♀, 1♂ from each litter were tested for elevated plus maze (PND 35), Morris water maze (PND 36), and openfield test (PND 34). Track 4: 1♀, 1♂ from each litter were tested for elevated plus maze (PND 70), Morris water maze (PND 71), and openfield test (PND 69).

Open-Field Test

The openfield apparatus was constructed of plastic (48 × 48 × 25 cm) and was divided into 8 × 8 cm squares. The box was warmed to nest temperature (33 C) by heating pads placed under the box. Lighting during the test was provided by two 15W fluorescent lights. Rats were placed individually in the open field and were monitored for 5 min. Testing was initiated by placing the rats in the center of the box. Each rat was placed in the same square facing the same direction for each testing period. A video camera was mounted above the box to record activity. Two individuals, who were blind to treatment, observed the video independently, and each recorded the number of squares crossed. The number of squares crossed with the four paws was used to measure locomotor activity. The two values obtained for each pup at each age were averaged to a single value. The amount of rearing activity was also considered. The box was cleaned with ethanol swabs between testing of the animals. This test was performed between 1900 h and 2300 h on PNDs 18, 20, 34, and 69.

Elevated Plus Maze

The test setting consists of a plusshaped apparatus, with two open (50 × 10 × 0.25 cm) and two closed (50 × 10 × 40 cm) arms emanating from a central platform (10 × 10 cm) and elevated 60 cm above the floor. All parts of the apparatus were made of dark polyvinyl plastic. Each rat was placed on the central platform facing one of the closed arms, and rats were allowed 5 min of free exploration in the apparatus. An entry into the arm was counted when the rat placed all four paws into the arm. Number of entries into the closed arm, the time spent in the open arms, the entries into the open arms, and latency to the first arm entry were recorded and analyzed. Rats were placed in the quiet experimental room for a minimum of 30 min prior to testing. Further, all testing sessions were directly observed on the video system by two investigators blinded to treatment conditions. This test was performed between 1900 h and 2300 h on PNDs 35 and 70.

Morris Water Maze

Rats exposed to BaP on PNDs 5–11 were observed at the age of 36 and 71 days for performance in a swim water maze. The swim maze, of Morris water maze type, was a circular pool, 130 cm in diameter, 50 cm in height, and filled with tap water at 22 C ± 1 C to a depth of 30 cm from the brim. The pool was rendered opaque by the addition of powdered milk. A circular escape platform 9 cm in diameter was placed 1.5 cm below the surface of the water in the middle of one of the four quadrants of the pool, and this position remained constant throughout testing. A video camera was mounted on the ceiling directly above the pool to record the swim path of each rat with a video tracking system. The room was illuminated by four 30W spotlights pointed at a white ceiling, and multiple distant cues around the room (window, cabinets, furniture, etc.) were kept in the same location throughout the experiments. Rats were placed in the quiet experimental room for a minimum of 30 min prior to testing. Rats were first acclimated to the maze during a onetrial habituation session, and all rats were first habituated to the maze with a 60s free swim in the pool without a platform. Hidden platform test began the day after habituation. For each trial, all animals were allowed to circumnavigate the pool in search of the escape platform for a maximum of 60 s. Hidden platform test was conducted for four trials per day on four consecutive days. Latencies to reach the escape platform were recorded. The animals were permitted 30 s to rest on the platform before removal from the pool. If a rat did not reach the platform within 60 s, it was gently guided to the platform by the experimenter. Rats remained on the platform for 30 s and were assigned a latency score of 60 s for that trial. Between the trials, the rats were toweled and fan dried and kept in holding cages for at least 5 min.

On the trial of day 5, the rats were given a 60s probe test of the spatial location with the platform absent. Rats were released from the quadrant opposite from the previous platform quadrant, and the swimming was tracked for 60 s. The number of times each animal crossed the original platform location and the time spent in the target quadrant were recorded. Testing was performed between 1900 h and 2300 h.

Statistical Analysis

The data were analyzed using parametric ANOVA (repeated measures ANOVA when appropriate). The linear model of such analysis included the following: treatment (four levels) and gender as betweensubject fixed factors; time as the repeated measures, withinsubject factor, for subjects. Further, body weight and behavioral measures were set as the dependant variables. *Post hoc* comparisons have been performed using the least significant difference (LSD) *t*test when ANOVAs were significant. Data were examined for the homogeneity of variance, and when the homogeneity of variance assumption was broken, data were analyzed by the KruskalWallis nonparametric analysis, followed by individual *post hoc* group comparisons. Significance was set at $p < 0.05$. Data were reported as mean ± SEM. All statistical analyses were carried out using the SPSS, version 17.0 (SPSS Inc.; Chicago, IL).

RESULTS

Maternal Behavior Observations

In the present study, no differences in any measures of maternal behaviors were observed following dosing of the pups.

Pup Body Weight

At the start of the experiments, the weight of pups was statistically equivalent across all groups ($F[3,76] = 0.32$, $p = 0.81$). No significant treatmentrelated changes in pup body weights were found during the 7day treatment period (refer to Fig. 1A). Throughout the 71day administration period, threeway ANOVA revealed significant effects for the three main factors on body weight—time: $F(8,648) = 72,161.07$, $p < 0.0001$; treatment: $F(3,648) = 35.71$, $p < 0.0001$; gender: $F(1,648) = 347.70$, $p < 0.0001$. The effect of postnatal exposure to BaP depended on age (treatment × time: $F[24,648] = 13.05$, $p < 0.0001$) but not on gender (treatment × gender: $F[3,648] = 1.82$, $p = 0.14$; treatment × time × gender: $F[24,648] = 0.46$, $p = 0.988$). A significant interaction between gender and age (gender × time: $F[8,648] = 211.09$, $p < 0.0001$) was also found. Given that the effect of BaP did not depend on gender, all rats were examined together. Thus, each day examined separately by oneway ANOVA analyses (PND 36: $F[3,76] = 24.38$, $p < 0.0001$; PND 71: $F[3,76] = 3.97$, $p = 0.01$), followed by *post hoc* comparisons, showed that pups exposed to 2 mg/kg BaP significantly gained less weight when compared with controls at PNDs 36 and 71 (Fig. 1B).

Developmental Landmarks

Eye Opening

In the present study, no significant differences were found for postnatal exposure to BaP (treatment: $F[3,72] = 0.57$, $p = 0.64$), gender (gender: $F[1,72] = 0.76$, $p = 0.39$), or their interaction (gender × treatment: $F[3,72] = 0.38$, $p = 0.77$) on the time of eye opening (shown in Fig. 2E).

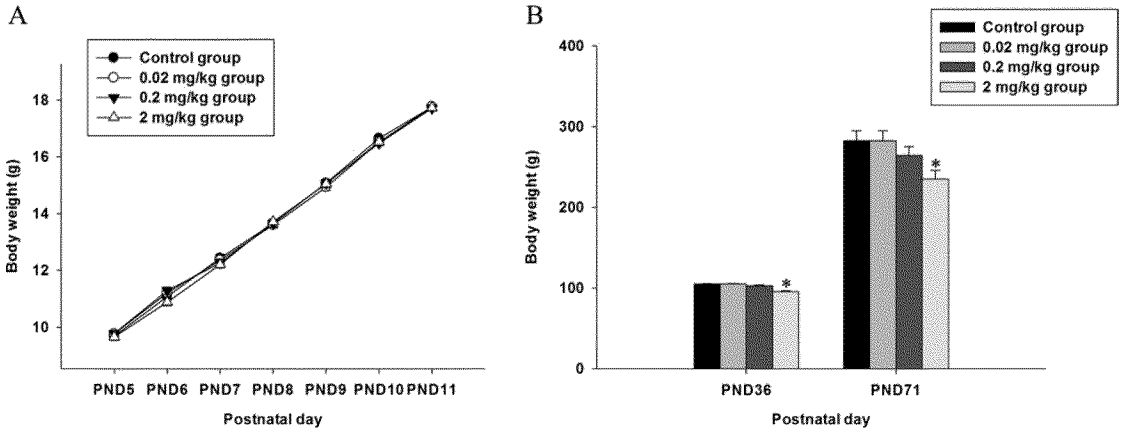

FIG. 1. Effects of postnatal exposure to BaP on the body weight. Values were expressed as mean ± SEM ($n = 20$). *$p < 0.05$ significantly different from controls. (A) No significant differences were found for postnatal exposure to BaP on pups' body weight during the 7day administration among four groups. (B) Interestingly, our experimental data showed that rats exposed to 2 mg/kg significantly gained less weight when compared with controls at PNDs 36 and 71.

Incisor Eruption

Statistical analyses showed that there were no significant differences for postnatal exposure to BaP (treatment: $F[3,72] = 0.39$, $p = 0.76$), gender (gender: $F[1,72] = 2.06$, $p = 0.16$), or their interaction (treatment × gender: $F[3,72] = 0.60$, $p = 0.62$) on the time of incisor eruption (Fig. 2D).

Development of Fur

Twoway ANOVA showed that there were no significant effects of BaP on the time of development of fur (treatment: $F[3,72] = 1.23$, $p = 0.31$). Moreover, the effect of postnatal exposure to BaP depended on gender (gender: $F[1,72] = 4.51$, $p = 0.04$). However, no interaction between these two factors

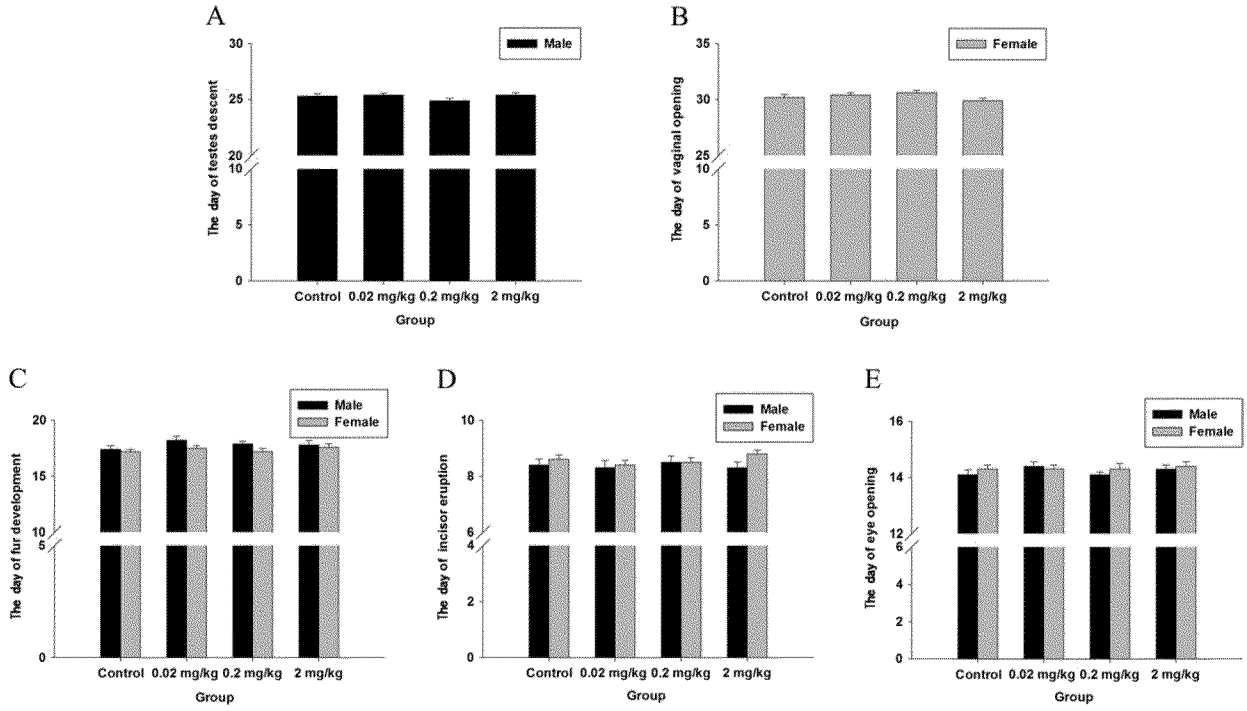

FIG. 2. Effects of postnatal exposure to BaP on the developmental landmarks. Values were expressed as mean ± SEM ($n = 10$). (A) No significant differences were found for postnatal exposure to BaP on the day of testes descent among four groups in the present study. (B) The day of vaginal opening did not differ significantly among treatment groups. (C) There were no significant differences among treatment groups on the day of fur development. (D) No significant differences were seen among treatment groups on the day of incisor eruption. (E) There were no significant group differences for the day of eye opening.

was found (treatment × gender: $F[3,72] = 0.46$, $p = 0.71$) (Fig. 2C).

Testes Descent

Oneway ANOVA revealed that there were no significant differences among treatment groups and controls for the time of testes descent ($F[3,36] = 1.29$, $p = 0.29$) (shown in Fig. 2A).

Vaginal Opening

As we showed in Figure 2B, statistical analyses did not reveal significant differences among treatment groups and controls for the time of vaginal opening ($F[3,36] = 1.67$, $p = 0.19$).

Neonatal Sensory and Motor Development

Surface Righting Reflex Test

Results revealed a significant effect of postnatal exposure to BaP on the righting reflex (treatment: $F[3,72] = 6.76$, $p = 0.0004$), depending on the age (treatment × time: $F[9,72] = 5.12$, $p < 0.0001$) but not on the gender (treatment × gender: $F[3,72] = 2.13$, $p = 0.10$). There were significant effects of age (time: $F[3,72] = 102.35$, $p < 0.0001$) but no significant effect of gender (gender: $F[1,72] = 3.19$, $p = 0.08$). Also, no interaction between these two factors was found (gender × time: $F[3,72] = 1.86$, $p = 0.14$). Moreover, the threeway repeated measure ANOVA revealed that there was a significant interaction effect in this test (treatment × gender × time: $F[9,72] = 2.52$, $p = 0.01$). Given that the effects of BaP administration did not depend on gender, all rats were examined together. Each day examined separately and analyzed by using oneway ANOVA (PND 12: $F[3,76] = 3.56$, $p = 0.02$; PND 14: $F[3,76] = 5.97$, $p = 0.001$; PND 16: $F[3,76] = 10.52$, $p < 0.0001$), followed by *post hoc* comparisons (LSD test), showed that only pups postnatally exposed to 0.2 mg/kg BaP needed significantly more time to place all four limbs under the body than controls at PND 12. On PND 14, these significant effects were found at both 0.02 and 2 mg/kg groups. Otherwise, on PND 16, significance was found only at the 2 mg/kg BaP-treated group (Fig. 3A). However, no significant effect was found at PND 18 ($F[3,76] = 2.21$, $p = 0.09$).

Negative Geotaxis Test

Significant effects of postnatal exposure to BaP on the negative geotaxis were found by threeway repeated measure ANOVA (treatment: $F[3,72] = 10.77$, $p < 0.0001$). These findings were verified for age (time: $F[3,72] = 73.92$, $p < 0.0001$). Also, results showed that effects of BaP depended on age (treatment × time: $F[9,72] = 2.67$, $p = 0.01$). However, the performance did not depend on the gender (gender: $F[1,72] = 1.33$, $p = 0.25$) or their interactions (treatment × gender: $F[3,288] = 0.37$, $p = 0.78$; gender × time: $F[3,288] = 0.30$, $p = 0.83$; treatment × time × gender: $F[9,72] = 0.57$, $p = 0.82$). Consequently, as the effect of treatment did not depend on gender, all animals were analyzed together by using oneway ANOVA analyses (PND 12: $F[3,76] = 7.74$, $p < 0.0001$; PND 14: $F[3,76] = 4.18$, $p = 0.009$; PND 16: $F[3,76] = 0.30$, $p = 0.83$; PND 18: $F[3,76] = 1.81$, $p = 0.15$), followed by *post hoc* comparisons; this revealed that on PND 12 significant effects were found at all three dose levels and in apparent dose-related fashion, whereas on PND 14 significant effects were found only at the 2 mg/kg dose when compared with controls (shown in Fig. 3B). In addition, the behavior exhibited by the rats such as falling, crawling off, or not moving resulted in the delayed latency in the negative geotaxis test.

Cliff Aversion

In the cliff aversion test, results showed that there were no significant effects of BaP (treatment: $F[3,72] = 0.65$, $p = 0.59$), gender (gender: $F[1,72] = 0.60$, $p = 0.44$), or their interaction (treatment × gender: $F[3,72] = 1.01$; $p = 0.39$) on the latency to retract the body from the edge of BaP-exposed rats (shown in Fig. 3C).

Forelimb Grip Strength

In the forelimb grip strength test, no significant effect was found for postnatal exposure to BaP on the grasping time by suspended rats (treatment: $F[3,72] = 0.76$, $p = 0.52$). Moreover, the performance did not depend on the gender (gender: $F[1,72] = 0.84$, $p = 0.36$) or their interaction (treatment × gender: $F[3,72] = 1.11$, $p = 0.35$) (Fig. 3D).

OpenField Test

In the present study, significant effects were found for treatment on the number of squares crossed by postnatal exposure to BaP (treatment: $F[3,288] = 7.67$, $p < 0.0001$). The performance depended on the age and gender (time: $F[3,288] = 114.8$, $p < 0.0001$; gender: $F[1,288] = 17.91$, $p < 0.0001$). Also, there were statistically significant differences on the interaction between treatment and age and between gender and age (treatment × time: $F[9,288] = 3.34$, $p = 0.001$; gender × time: $F[3,288] = 3.68$, $p = 0.013$). However, no statistically significant interactions were found between treatment and gender and three variables (treatment × gender: $F[3,288] = 0.50$, $p = 0.69$; treatment × time × gender: $F[9,288] = 0.50$, $p = 0.87$). Thus, all rats were examined together by using one-way ANOVA (PND 18: $F[3,76] = 0.14$, $p = 0.93$; PND 20: $F[3,76] = 0.07$, $p = 0.98$; PND 34: $F[3,76] = 2.79$, $p = 0.046$; PND 69: $F[3,76] = 12.23$, $p < 0.0001$). Further, *post hoc* comparisons revealed that pups exposed to BaP showed a significant increase on the locomotor activity. Pups exposed to 2 mg/kg BaP significantly increase the number of squares crossed than controls at PND 34. However, at PND 69, both 0.2 and 2 mg/kg groups showed a more significant increase in locomotor activity than controls (Fig. 4A).

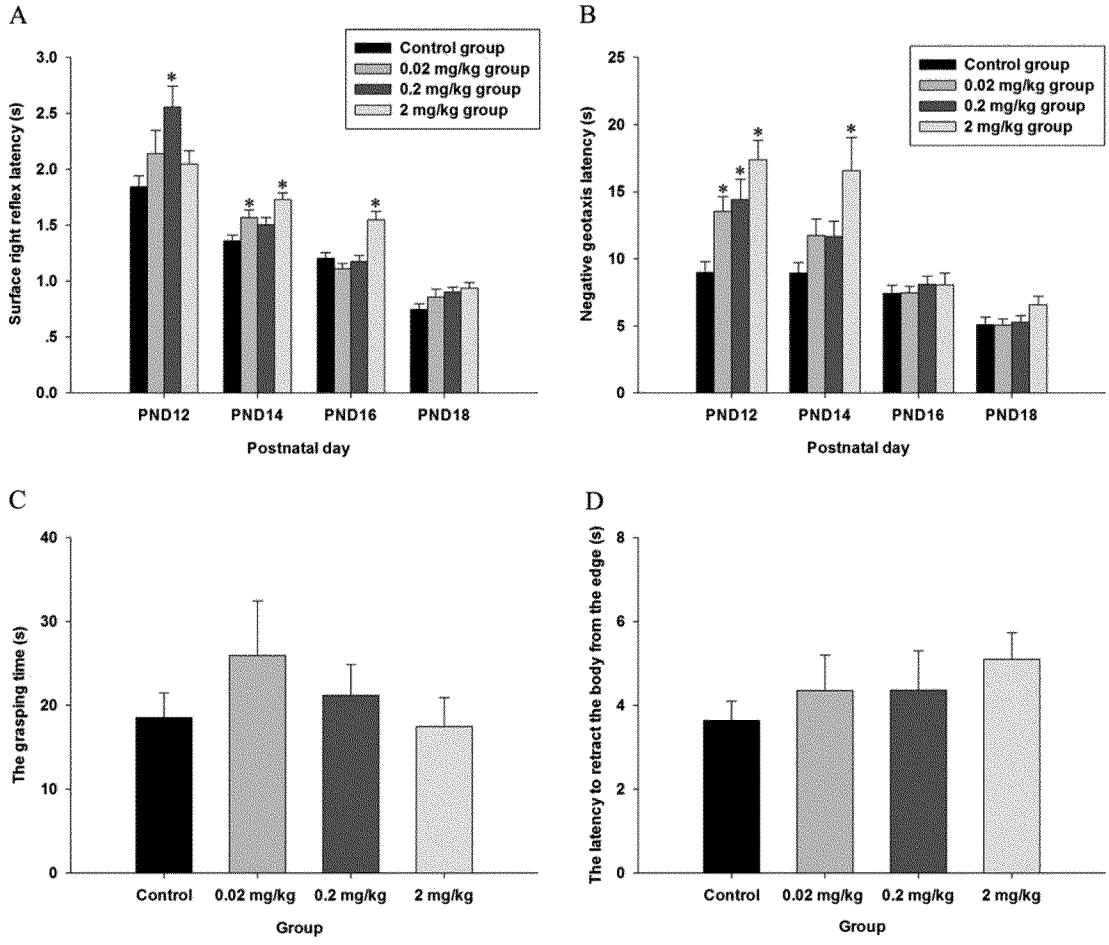

FIG. 3. Effects of postnatal exposure to BaP on the neonatal sensory and motor development. Data were expressed as mean ± SEM ($n = 20$). *$p < 0.05$ significantly different from controls. (A) In the surface righting reflex test, results revealed a significant effect of postnatal exposure to BaP on the righting reflex. (B) Significant effects of postnatal exposure to BaP on the negative geotaxis were found by threeway ANOVA. (C) There were no significant differences among treatment groups on the grasping time by suspended rats at PND 12 in the forelimb grip strength test. (D) Twoway ANOVA revealed that no significant differences were seen among four groups on the latency to retract the body from the edge in the cliff aversion test at PND 12.

Concerning rearing, threeway ANOVA revealed that there were significant effects of BaP (treatment: $F[3,288] = 4.01$, $p = 0.008$) on the vertical locomotor activity. These results were verified for age (time: $F[3,288] = 59.97$, $p < 0.0001$) and gender (gender: $F[1,288] = 4.01$, $p = 0.046$). However, the effects of postnatal exposure to BaP did not depend on age (treatment × time: $F[9,288] = 1.56$, $p = 0.12$), gender (treatment × gender: $F[3,288] = 0.80$, $p = 0.49$), or their interaction (time × gender: $F[3,288] = 0.91$, $p = 0.43$; treatment × time × gender: $F[9,288] = 0.80$, $p = 0.62$) for the rearing activity. Thus, all animals were examined together by using the oneway ANOVA analyses (PND 18: $F[3,76] = 0.06$, $p = 0.98$; PND 20: $F[3,76] = 0.17$, $p = 0.92$; PND 34: $F[3,76] = 1.99$, $p = 0.12$; PND 69: $F[3,76] = 4.19$, $p = 0.008$), followed by LSD test. Results showed that animals exposed to 2 mg/kg BaP exhibited significantly more locomotor activity than control pups at PND 69 (Fig. 4B).

However, there were no statistically significant differences between groups in any measure of locomotor activity when tested at both 18 and 20 days of age.

Elevated Plus Maze

In the elevated plus maze, our results revealed a significant effect of postnatal exposure to BaP on the latency time of the first entry into an open arm (treatment: $F[3,144] = 10.27$, $p < 0.0001$). This effect of treatment depended on age (treatment × time: $F[3,144] = 18.88$, $p < 0.0001$). However, this performance did not depend on the age (time: $F[1,144] = 0.08$, $p = 0.78$), gender (gender: $F[1,144] = 0.87$, $p = 0.35$), or their interactions (gender × time: $F[1,144] = 0.32$, $p = 0.57$; treatment × gender: $F[3,144] = 0.40$, $p = 0.76$; treatment × time × gender: $F[3,144] = 1.10$, $p = 0.35$). Therefore, oneway ANOVA analysis (PND 35—male:

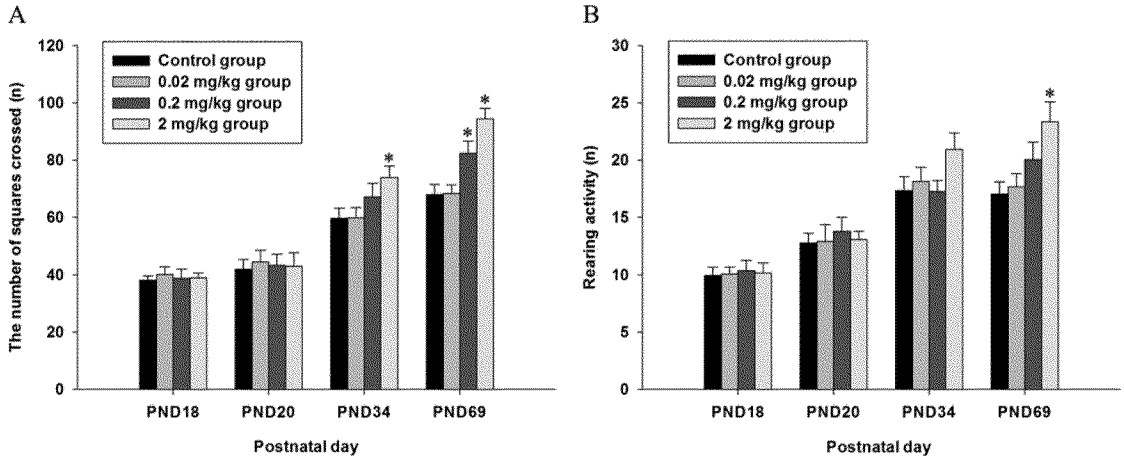

FIG. 4. Effects of postnatal exposure to BaP in the open field on PNDs 18, 20, 34, and 69. Data were expressed as mean ± SEM ($n = 20$). *$p < 0.05$ significantly different from controls. (A) Threeway ANOVA showed the significant effects of treatment on the number of squares traveled. (B) Threeway ANOVA revealed the significant effects of BaP on the rearing activity in the openfield test.

$F[3,36] = 1.12$, $p = 0.36$; female: $F[3,36] = 0.22$, $p = 0.89$; PND 70—male: $F[3,36] = 22.24$, $p < 0.0001$; female: $F[3,36] = 8.78$, $p < 0.0001$), followed by *post hoc* comparisons, showed that both male and female pups exposed to 0.2 and 2 mg/kg BaP took significantly less time to perform the first entry into an open arm than controls at PND 70 (Fig. 5A). However, no statistically significant differences were detected at PND 35.

Concerning the time spent in the open arm, threeway ANOVA revealed significant effects for treatment (treatment: $F[3,144] = 35.34$, $p < 0.0001$), a trend toward a gender effect (gender: $F[1,144] = 25.82$, $p < 0.0001$; treatment × gender: $F[3,144] = 6.54$, $p < 0.0001$) but not for the age (time: $F[1,144] = 0.93$, $p = 0.34$). Additionally, no interaction among the three variables was found in our study (gender × time: $F[1,144] = 1.61$, $p = 0.21$; treatment × time × gender: $F[3,144] = 0.84$, $p = 0.48$; treatment × time: $F[3,144] = 2.26$, $p = 0.08$). Therefore, each gender per day was analyzed separately by oneway ANOVA or KruskalWallis nonparametric analysis when appropriate (PND 35—male: $F[3,36] = 0.53$, $p = 0.67$; female: $F[3,36] = 13.60$, $p < 0.0001$; PND 70—male: $\chi^2_{(3)} = 9.57$, $p < 0.0001$; female: $F[3,36] = 22.49$, $p < 0.0001$). *Post hoc* analysis showed that male rats treated with BaP at 0.2 and 2 mg/kg spent significantly more time in the open arms than controls at PND 70. However, female rats exposed to 2 mg/kg BaP showed a significant increase in the time spent in the open arms at PND 35. Also, at PND 70, female rats exposed to three doses of BaP all showed a significant increase in the time spent in the open arms (Fig. 5B). However, there were no statistically significant differences for male rats at PND 35.

Threeway ANOVA revealed a significant main effect for postnatal exposure to BaP on the number of entries into the open arms (treatment: $F[3,144] = 11.39$, $p < 0.0001$). The effect was found for gender and age (gender: $F[1,144] = 11.89$, $p = 0.001$; time: $F[1,144] = 7.37$, $p = 0.007$). No significant effects were found for their interaction on the entries into the open arms (gender × time: $F[1,144] = 1.21$, $p = 0.27$; treatment × time × gender: $F[3,144] = 1.06$, $p = 0.37$). However, the effect of treatment did not depend on gender (treatment × gender: $F[3,144] = 0.33$, $p = 0.81$) but depended on the age (treatment × time: $F[3,144] = 3.53$, $p = 0.02$). Therefore, oneway ANOVA analysis (PND 35—male: $F[3,36] = 1.50$, $p = 0.23$; female: $F[3,36] = 0.10$, $p = 0.96$; PND 70—male: $F[3,36] = 7.38$, $p = 0.001$; female: $F[3,36] = 9.80$, $p < 0.0001$), followed by *post hoc* comparisons, showed that both male and female rats orally exposed to 2 mg/kg BaP made significantly more entries into the open arm than controls at PND 70. In addition, in the 0.2 mg/kg BaP group, female pups also made more entries into the open arms when compared with controls at PND 70 (Fig. 5C). However, no statistically significant differences were detected for either male or female rats at PND 35.

With regard to the number of entries into the closed arms, threeway ANOVA revealed a significant effect of BaP (treatment: $F[3,144] = 13.85$, $p < 0.0001$) but not for gender (gender: $F[1,144] = 1.39$, $p = 0.24$) or age (time: $F[1,144] = 0.08$, $p = 0.78$). Moreover, the interactions among these three variables were also not significant (gender × time: $F[1,144] = 0.45$, $p = 0.50$; treatment × gender: $F[3,144] = 1.50$, $p = 0.22$; treatment × time × gender: $F[3,144] = 1.06$, $p = 0.37$). However, the effect of treatment depended on the age (treatment × time: $F[3,144] = 2.99$, $p = 0.03$). Oneway ANOVA (PND 35—male: $F[3,36] = 2.69$, $p = 0.06$; female: $F[3,36] = 1.50$, $p = 0.23$; PND 70—male: $F[3,36] = 3.71$, $p = 0.02$; female: $F[3,36] = 11.09$, $p < 0.0001$) followed by *post hoc* comparison analysis showed that male pups treated with 2 mg/kg BaP made fewer entries into the closed arms than controls at PND 70. However, female pups exposed to 0.2 and 2 mg/kg BaP made significantly fewer entries into the closed

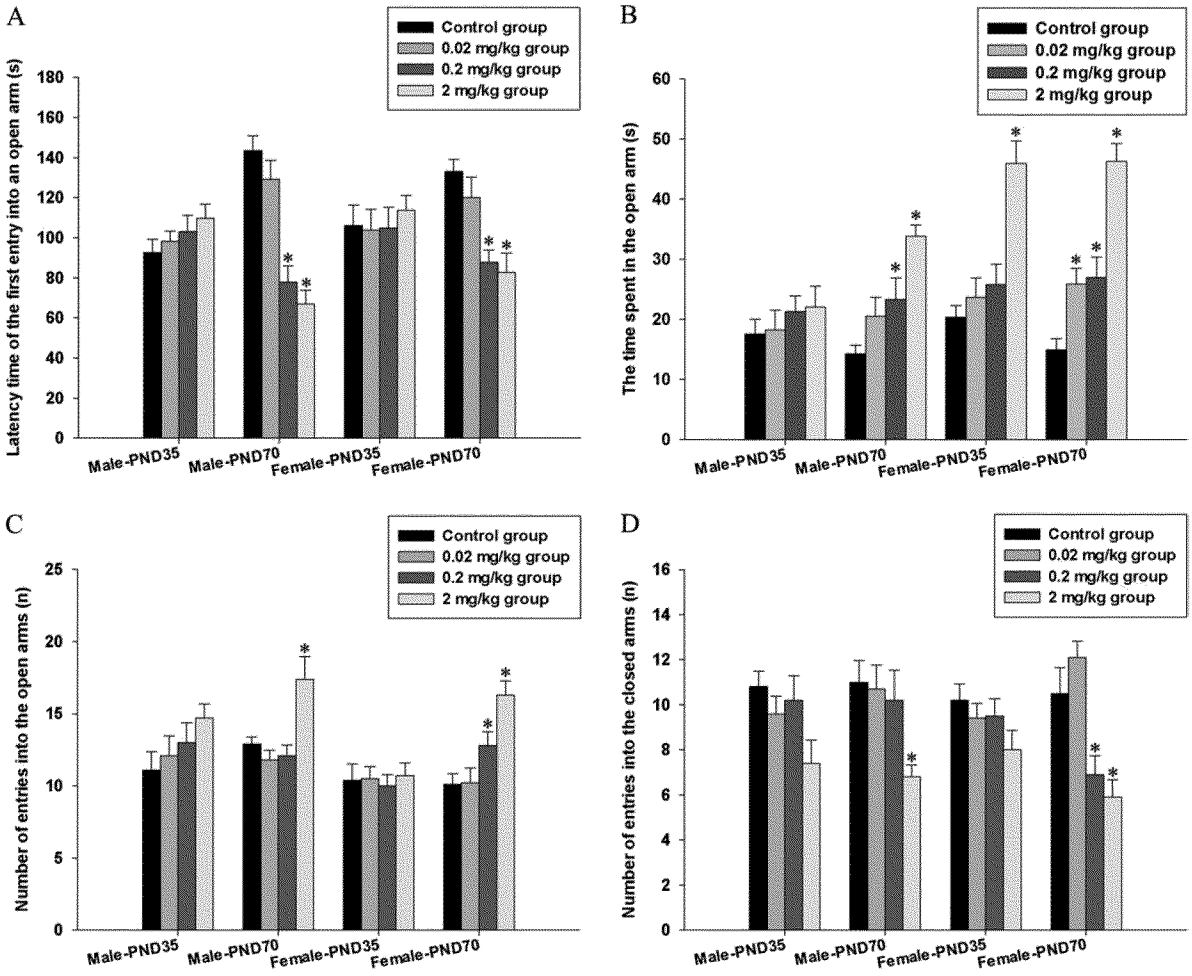

FIG. 5. Effects of postnatal exposure to BaP in the elevated plus maze on PNDs 35 and 70. Data were reported as mean ± SEM ($n = 10$). *$p < 0.05$ significantly different from controls. (A) Results revealed a significant effect of postnatal exposure to BaP on the latency time of the first entry into an open arm. (B) Threeway ANOVA revealed significant effects for treatment on the time spent in the open arm. (C) Results demonstrated that there were significant effects of BaP on the number of entries into the open arms. (D) Our data showed a significant effect of BaP on the number of entries into the closed arms.

arms than controls at PND 70 (Fig. 5D). Moreover, there were also no statistically significant treatment differences for both male and female pups at PND 35.

Morris Water Maze

During the hidden platform trials in the water maze, three-way repeated measure ANOVA revealed that significant effects were found for postnatal exposure to BaP on the escape latency at juvenile test period (treatment: $F[3,72] = 57.62$, $p < 0.0001$). However, the effect of treatment did not depend on gender (gender: $F[1,72] = 0.04$, $p = 0.85$; treatment × gender: $F[3,72] = 0.06$, $p = 0.98$) or interactions (gender × time: $F[3,72] = 0.17$, $p = 0.91$; treatment × time: $F[9,72] = 1.37$, $p = 0.22$) but depended on the age (time: $F[3,72] = 82.84$, $p < 0.0001$). LSD *t*test showed that rats exposed to 2 mg/kg BaP needed more time to find the platform than controls at juvenile test period (Figs. 6A and 6B). Also, at adult test periods, three-way repeated measure ANOVA revealed a significant effect of BaP (treatment: $F[3,72] = 86.35$, $p < 0.0001$) on the escape latency; however, the effect of BaP did not depend on the gender (gender: $F[1,72] = 0.25$, $p = 0.62$) or their interactions (treatment × gender: $F[3,72] = 0.11$, $p = 0.95$; treatment × time: $F[9,72] = 0.53$, $p = 0.84$; gender × time: $F[3,72] = 0.06$, $p = 0.98$). *Post hoc* comparisons showed that in rats exposed to the 0.2 and 2 mg/kg dose level of BaP, the escape latencies were significantly increased at adult test period (Figs. 6C and 6D).

During the probe trial test in the water maze, significant effects were found for postnatal exposure to BaP on the performance made by pups in Morris water maze test (treatment: $F[3,144] = 19.77$, $p < 0.0001$). This effect was verified for age and gender (time: $F[1,144] = 6.04$, $p = 0.02$; gender: $F[1,144] = 5.48$, $p = 0.02$). The effects of postnatal exposure to BaP did not depended on age (treatment × time: $F[3,144] = 0.74$, $p = 0.53$) or gender (treatment × gender:

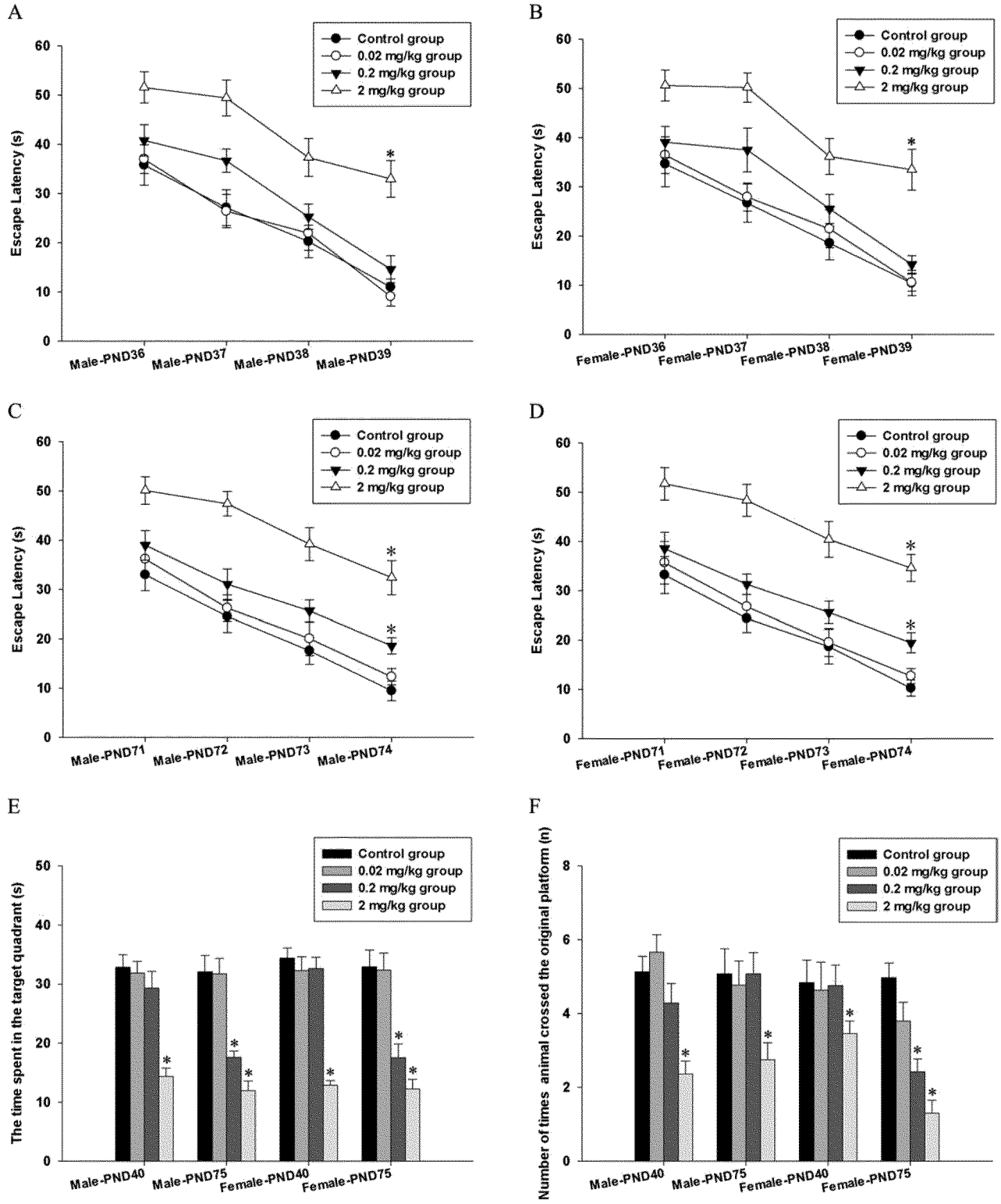

FIG. 6. Effects of postnatal exposure to BaP in the Morris water maze at adolescent and adult test periods. Data were reported as mean ± SEM ($n = 10$). *$p < 0.05$ significantly different from controls. (A) There were significant effects of treatment on the escape latency in male rats at adolescent test time. (B) At adolescent test time, the significant effects of BaP on the escape latency were found in female rats. (C) There were significant effects of BaP on the escape latency in male rats at adult test period. (D) At adult test time, the significant effects of treatment on the escape latency were found in female rats. (E) Our data showed there were significant effects of BaP on the number of times the animal crossed the original platform in the probe test. (F) There were significant effects of treatment on the time spent in the target quadrant.

$F[3,144] = 0.93$, $p = 0.43$). However, the interactions among these variables were also significant (gender × time: $F[1,144] = 6.72$, $p = 0.01$; treatment × time × gender: $F[3,144] = 2.72$, $p = 0.04$). Consequently, each day analyzed separately by using oneway ANOVA (PND 40: male: $F[3,36] = 10.36$, $p < 0.0001$; female: $F[3,36] = 1.24$, $p = 0.31$; PND 75: male: $F[3,36] = 3.53$, $p = 0.02$; female: $F[3,36] = 15.44$, $p < 0.0001$), followed by *post hoc* comparisons, revealed that male rats exposed to 2 mg/kg BaP showed a significant decrease in the number of times each animal crossed the original platform at PNDs 40 and 75. However, a significant decrease in the number of times the animal crossed the original platform was found both in the 0.2 and 2 mg/kg groups for female pups at PND 75 (Fig. 6F). There were no statistically significant treatment differences for female rats at PND 40.

Threeway ANOVA revealed main effect for postnatal exposure to BaP on the time spent in the target quadrant (treatment: $F[3,144] = 76.16$, $p < 0.0001$), and main effect of age (time: $F[1,144] = 14.13$, $p < 0.0001$) but no main effect of gender (gender: $F[1,144] = 0.42$, $p = 0.52$). The interactions among these three variables were not significant (gender × time: $F[1,144] = 0.06$, $p = 0.80$; treatment × gender: $F[3,144] = 0.23$, $p = 0.88$; treatment × time × gender: $F[3,144] = 0.26$, $p = 0.86$). However, the interaction between treatment and age was significant (treatment × time: $F[3,144] = 8.61$, $p < 0.0001$). Oneway ANOVA (PND 40—male: $F[3,36] = 16.06$, $p < 0.0001$; female: $F[3,36] = 33.78$, $p < 0.0001$; PND 75—male: $F[3,36] = 22.71$, $p < 0.0001$; female: $F[3,36] = 17.98$, $p < 0.0001$), followed by *post hoc* analyses, showed that both male and female rats exposed to 2 mg/kg BaP spent significantly less time in the target quadrant at PND 40. However, the results showed that male and female rats treated with 0.2 and 2 mg/kg BaP showed significant decrease in the time spent in the target quadrant at PND 75 (Fig. 6E).

In addition, during the probe trial, onefactor ANOVA indicated no significant difference in swim speed among groups both at juvenile and adult test periods (juvenile—male: $F[3,36] = 0.32$, $p = 0.81$; female: $F[3,36] = 0.44$, $p = 0.72$; adult—male: $F[3,36] = 0.29$, $p = 0.83$; female: $F[3,36] = 0.10$, $p = 0.96$).

DISCUSSION

Over the past decades, numerous studies have assessed the potential neurotoxic effects of either acute or chronic exposure to BaP (Bouayed *et al.*, 2009; Niu *et al.*, 2010; Saunders *et al.*, 2001; Wormley *et al.*, 2004; Xia *et al.*, 2011). However, to our knowledge, this is the first report documenting the persistent behavioral neurotoxic effects of repeated oral doses of BaP on the developing brain. Our results suggest that postnatal exposure to BaP during a critical phase of neonatal brain development can cause neurobehavioral impairments that become increasingly apparent with the progression to adulthood. These current findings provide evidence that neural and behavioral impairments resulting from postnatal BaP exposure are potentially longlasting, may not be apparent in juveniles, but present in adolescence and young adulthood.

Previous studies have characterized acute or chronic lowdose neurobehavioral effects in response to other toxicants at early postnatal times (Karpova *et al.*, 2009; Tchekalarova *et al.*, 2005; Tseng *et al.*, 2006). However, the objective of this study was to investigate the persistent effects of BaP in rats after exposure during the phase of neonatal brain development. The time point selected in this study was made to be more comparable with the fetal period of human development. During this period, the neuronal proliferation is completed in most brain regions with the exception of the dentate gyrus and cerebellum (Kuhn *et al.*, 1996). Our findings indicated that early postnatal exposure to BaP could result in the delayed neurobehavioral effects that were evident later in life. These results support the evidences that postnatal BaP exposure leads to altered motor activity, emotional, and cognitive function in the adolescent and adult, and these adverse effects are longlasting and may worsen with age. There are several possible explanations for why BaP would induce an earlier onset and exert longerlasting harmful effects on these behavioral endpoints. This may be due to pharmacokinetic factors such as low rates of metabolism and excretion, increased storage in fatty tissue, and differences in plasma protein binding characteristics (Das *et al.*, 1985; Schuler *et al.*, 2003; Stavric and Klassen, 1994; Tipping *et al.*, 1980). However, the underlying cellular mechanisms for the phenomenon were not investigated in the present study and will be the focus of future studies. In addition, the "withinlitter design" is used in the present study. Thus, pups within a litter have different treatments. In this case, the procedure can minimize the effects of maternal influence, give an even distribution of littermates, and reduce the animal usage. However, this study design increases the risk of crosseontamination among groups, and untreated controls may also dominate the litter, and/or treated rats may be weak and subsequently rejected by the dams.

Although acute lowdose exposure to BaP in the present study did not affect early developmental milestones measured, delayed body weight gain was seen in rats exposed to 2 mg/kg BaP at PNDs 36 and 71, suggesting an adverse effect on somatic growth, possibly due to the impairments on endocrine function (Chung *et al.*, 2007). Further, in the surface righting reflex test, our data showed that pups postnatally exposed to BaP needed significantly more time to place all four limbs under the body than controls at PNDs 12, 14, and 16. Also, rats exposed to BaP showed a significant increase in the latency time at PNDs 12 and 14 when compared with controls in the negative geotaxis test. However, in both the cliff aversion and forelimb grip strength tests, no significant effects were found for postnatal exposure to BaP. Many of the behavioral alterations reported here were significant only in the middle- and/or highdose groups, indicating a lack of doseresponse. Taken together, these

findings suggest subtle changes in neonatal sensory and motor development following exposure to BaP.

In this study, no significant alteration of motor activity was found in the BaP-exposed pups at PNDs 18 and 20. These results were in agreement with the previous study that treated with BaP lactationally (Bouayed *et al.*, 2009). The lack of behavior deficits in the preweaning pups was understandable, considering the low activity of rats of these ages. However, our results showed that the 0.2 and 2 mg/kg BaP dose caused significant locomotor activity deficits in rats tested as juvenile stage or adulthood in the open-field test. The locomotor activity deficits in BaP-treated animals were evident as a trend at PND 34 and were most pronounced at PND 69. This suggested that developmental exposure to BaP could result in long-lasting deficits in the locomotor activity. Also, increased rearing was observed during open-field test in animals exposed to 2 mg/kg BaP at PND 69. Beginning at the PND 34 open-field test, rearing had already increased; however, no dose groups attained statistical significance. By PND 69, rearing activity was significantly increased in the high-dose group. This finding suggested a progression of effect with age. One possible explanation for the phenomenon may be due to the hippocampal lesions. Increased locomotor activity after hippocampal lesions is a well-established phenomenon (Iuvone and Van Hartesveldt, 1976). Also, a previous study has demonstrated that BaP exposure can cause alterations in the dopaminergic and monoaminergic systems throughout the brain, especially in the hippocampus (Xia *et al.*, 2011; Zhang *et al.*, 2008).

The elevated plus maze test is a widely used animal test for anxiety. However, to date, no one has yet explored persistent neurotoxic effects of postnatal BaP exposure by using a plus maze. In the current study, our data demonstrated that postnatal exposure to BaP resulted in several behavioral alterations that characterize an anxiolytic-like action both in adolescent and adult rats. Exposure to BaP produced a significant increase in open arm entries and the time spent in open spaces of the elevated plus maze. Also, results showed a significant decrease in the closed arm entries of pups that were orally exposed to BaP. Moreover, these measures in the elevated plus maze had no evident effect on adolescent rats but greater anxiolytic effect in adult rats. These current findings suggest that the detrimental effects of postnatal BaP exposure may emerge following adolescence. The potential reasons for these delayed neurotoxic effects are unknown at this time. Also, in this study, several behavioral measures in the elevated plus maze showed significant differences between males and females. Additionally, sex differences have not been noted previously following BaP exposure. These data indicated that female might be more sensitive to BaP developmental neurotoxicity than male. The detrimental effects in females may be due to the different metabolic rates of BaP (Knuckles *et al.*, 2001). Furthermore, differences in metabolic enzyme levels regulated by hormones may also account for the greater sensitivity of females in this behavioral endpoint (Ramesh *et al.*, 2000). Another possible explanation for these results may be due to the hormonal state at the time of testing.

The present study demonstrated that acute postnatal administration of BaP induced spatial learning deficits in swim maze. In the water maze, the BaP-exposed rats required more time to escape the maze during the four training sessions. Also, rats exposed to BaP showed a significant decrease in both the number of times the platform was crossed and the time spent in the target quadrant. Further, at the juvenile swim maze test period, the spatial learning deficits only showed in the high-dose group. However, these impairments emerged both in the middle- and high-dose groups at adult test period. To our knowledge, animal performances in the water mazes are related to the integrity of hippocampus and spatial memory function (Zola-Morgan and Squire, 1990). Rats exposed to BaP may suffer from severe neuronal damage, expressed as a variety of behavioral problems, including spatial learning deficits. Previous study from our laboratory has confirmed that BaP exposure can result in abnormal neuronal alterations in the hippocampus (Chen *et al.*, 2011). The dose levels used in the present study correspond to the levels of contamination in smokers, heavy consumers of smoked or grilled meat and fish, individuals who live in the heavy PAH-polluted areas, etc. (ATSDR, 1995). Thus, the results observed in the swim maze suggest that environmental doses of postnatal BaP exposure can impair spatial learning functions at juvenile stage and worsen at young adulthood.

The biological basis for BaP-induced neurobehavioral toxicity is as yet unclear. Some previous studies have proposed that BaP neurotoxic effects are due to the severe levels of oxidative stress (Gao *et al.*, 2011; Murawska-Ciałowicz *et al.*, 2011). Indeed, oxidative stress plays a key role in the development of the brain, including neurotransmission, and causes neuronal cell death. Furthermore, recent studies by Saunders *et al.* (2006) showed that acute exposure to BaP caused a significant decrease in levels of superoxide dismutase and glutathione peroxidase, as well as enhancement in catalase and lipid peroxidation. Another potential explanation for BaP developmental neurotoxicity involves interactions with neurotransmitter systems. Alterations in the dopaminergic and monoaminergic systems throughout the brain have been reported after BaP exposure (Nie *et al.*, 2008; Xia *et al.*, 2011; Zhang *et al.*, 2008). These findings also support the hypothesis that PAHs can cause tissue damage in the central nervous system. It is well known that the hippocampus plays an essential role in spatial learning and memory processes in animals. Thus, the alternation in the neurotransmitter systems in the hippocampus may contribute to the impairment of learning and memory. Furthermore, Grova *et al.* (2007, 2008) have reported changes in N-Methyl-D-aspartate receptors in the hippocampus following subacute exposure to BaP. BaP exposure has been shown to reduce long-term potentiation in the hippocampus of exposed mice, possibly by altering the expression of glutamate receptor subunits (Brown *et al.*, 2007; Wormley *et al.*, 2004). Additionally, BaP has direct actions on

developing neuronal cells that could contribute to the adverse neurodevelopmental effects seen with *in vivo* exposures. Previous studies have shown that BaP is capable of causing protein kinase C inhibition, alter calcium signaling patterns, and induce impairments in cultured neuronal cells (Barhoumi *et al.*, 2002; Ou and Ramos, 1994; Ou *et al.*, 1995; Slotkin and Seidler, 2009). Also, BaP-induced changes in messenger RNA expression may underlie some of these neurotoxic effects (Dong *et al.*, 2008; Wang *et al.*, 2006). However, to date, the mechanisms are still unknown.

In summary, postnatal exposure to BaP during neonatal brain development can cause delayed neurobehavioral impairments. The results provide additional evidence for developmental neurotoxicity of BaP. Also, given the widespread and pronounced exposure of infants and children to PAHs, these data indicate the need for further mechanistic studies to understand the potential for developmental neurotoxicity of PAHs in the humans.

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